Identification of the Antiarrhythmic Drugs Amiodarone and Lorcainide as Potent H3 Histamine Receptor Inverse Agonists

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ABSTRACT

Use of molecular pharmacology to reprofile older drugs discovered before the advent of recombinant technologies is a fruitful method to elucidate mechanisms of drug action, expand understanding of structure-activity relationships between drugs and receptors, and in some cases, repurpose approved drugs. The H3 histamine receptor is a G-protein-coupled receptor (GPCR) primarily expressed in the central nervous system wherein many things it modulates cognitive processes, nociception, feeding and drinking behavior, and sleep/wakefulness. In binding assays and functional screens of the H3 histamine receptor, the antiarrhythmic drugs lorcainide and amiodarone were identified as potent, selective antagonists/inverse agonists of human and rat H3 histamine receptors, with relatively little or no activity at over 20 other monoamine GPCRs, including H1, H2, and H4 receptors. Potent antagonism of H3 receptors was unique to amiodarone and lorcainide of 20 antiarrhythmic drugs tested, representing six pharmacological classes. These results expand the pharmacophore of H3 histamine receptor antagonist/inverse agonists and may explain, in part, the effects of lorcainide on sleep in humans.

Introduction

The existence of a third histamine receptor subtype was first proposed in 1983 based on the observation that histamine could inhibit its own release from neurons in cortical brain slices with a pharmacology that deviated from known H1 and H2 receptor pharmacology (Arrang et al., 1983). Subsequent discovery of H3-selective ligands (Arrang et al., 1987), followed by the cloning of the H3 receptor (Lovenberg et al., 1999), has greatly facilitated research into the physiologic functions of this receptor.

The H3 receptor couples to Goi G-proteins to inhibit cAMP production and is expressed primarily in the central nervous system (Lovenberg et al., 1999), where it acts as an autoreceptor, controlling histamine release as well as the release of other neurotransmitters including acetylcholine, dopamine, GABA, 5-hydroxytryptamine, and peptides (Haas et al., 2008). The H3 receptor undergoes extensive splicing to produce a number of distinct isoforms (Bakker, 2004), which have distinct pharmacological properties (Cegé et al., 2001; Wellendorph et al., 2002). The H3 receptor displays a high degree of constitutive activity in vivo and this tonic activity is likely an important feature contributing to its control of neurotransmitter release (Morisset et al., 2000). For example, reduction in the tonic activity of H3 autoreceptors increases histamine release that stimulates postsynaptic H1 histamine receptors, leading to increased wakefulness. Due to its role in modulating the activities of many important neurotransmitter systems, ligands that modulate H3 receptor activity affect central nervous system control of cognitive processing, nociception, feeding and drinking behavior, and sleep/wakefulness. H3 histamine receptor antagonists/inverse agonists are being tested clinically as therapeutic agents to treat narcolepsy, cognitive impairment in Alzheimer’s disease, schizophrenia, and obesity, among others (Passani and Blandina, 2011).

Lorcainide and amiodarone are two structurally and pharmacologically distinct antiarrhythmic drugs. Lorcainide is a class IC antiarrhythmic that works by blocking open fast acting voltage-gated sodium channels (subtype Na1.5), slowing the upstroke of ventricular myocyte action potential (Amery et al., 1981). Lorcainide is used to treat patients with premature ventricular contractions and Wolff-Parkinson-White syndrome (Samánek et al., 1987). Disturbed sleep is a prominent side effect of lorcainide usage [see Eiriksson and Brogden (1984)]. Amiodarone is a class III antiarrhythmic that prolongs the repolarization phase of the cardiac action potential by blocking potassium channels (Rosenbaum et al., 1983). Although amiodarone is an effective antiarrhythmic agent, it has side effects on a number of organ systems, limiting its use (Santangeli et al., 2012).

To better understand the molecular basis for the clinical actions of drugs, we have been systematically screening and profiling collections of clinically used compounds for activity in functional assays using heterologously expressed G-protein-coupled receptors (GPCRs). Using a cellular proliferation assay called receptor selection and amplification technology (R-SAT) (Burstein et al., 2006), we identified lorcainide and amiodarone from a diverse collection of antiarrhythmic drugs as potent H3 receptor inverse agonists and may explain, in part, the effects of lorcainide on sleep in humans.
histamine receptor antagonists. Further profiling revealed that both agents display inverse agonist activity at certain H3 receptor isoforms, as well as surprising selectivity for H3 receptors over other GPCRs.

Materials and Methods

NIH-3T3 cells (CRL 1658) and HEK-293T cells (CRL 11268) were purchased from American Type Culture Collection (Manassas, VA). O-Nitrophenyl-β-D-galactopyranoside and Nonidet P-40 were from Sigma-Aldrich (St. Louis, MO). Dulbecco’s modified Eagle’s medium (DMEM) (Gibco-BRL, Grand Island, NY) was used for tissue culture in 96-well Falcon tissue culture plates (Thermo Fisher Scientific Inc., Waltham, MA). Hanks’ balanced salt solution (not containing magnesium chloride, magnesium sulfate, or calcium chloride) and trypsin-EDTA were both from Gibco-BRL.

Drugs. All compounds were solubilized as 10 mM stock solutions in dimethylsulfoxide (DMSO), and subsequently diluted into working solutions with the appropriate buffer. DMSO concentration was never greater than 0.5% at the top concentration of any working solution, a DMEM concentration that we have found does not affect assay performance. Working dilutions were made in the appropriate assay buffers. All compounds were obtained from Tocris Bioscience (Bristol, UK) or Sigma/RBI (St. Louis, MO).

Cell Culture. NIH-3T3 cells were incubated at 37°C in a humidified atmosphere (5% CO2) in DMEM with the same supplements used for NIH-3T3 cells except that plus 10% fetal calf serum was used instead of calf serum. HEK-293T cells were incubated at 37°C in a humidified atmosphere (5% CO2) in DMEM supplemented with 25% Ultraculture synthetic supplement (Cambrex, Walkersville, MD) instead of calf serum to a final volume of 200 µl/well. After 5 days in culture, β-galactosidase activity was measured and responses were quantified on a BioTek EL 310 (Winooski, VT) or Molecular Devices (Sunnyvale, CA) plate reader. The data for 5-HT1A, 5-HT1B, 5-HT1D, 5-HT1E, 5-HT1F, 5-HT2A, 5-HT2B, 5-HT2C(vgv), 5-HT7, α1A, α1B, α2A, α2B, α2C, M1, M2, M3, M4, and H1 receptors was generated using a similar method using cells grown in larger volumes (632 cm² cell factory flasks; Nalgene Nunc International, Rochester, NY), transfected, and frozen at −135°C until use as described previously (Burstein et al., 2011).

Conducts. Human D2 (short form), 5-HT1A, 5-HT1B, 5-HT1D, 5-HT1E, 5-HT1F, 5-HT2A, 5-HT2B, 5-HT2C(vgv), 5-HT7, α1A, α1B, α2A, α2B, α2C, M1, M2, M3, M4, M5, and H3 receptors were described previously (Wellendorph et al., 2002; Burstein et al., 2011). The human H2 and H4 receptors and the rat ortholog of isoform 1 of the human H3 receptor were cloned by Burstein et al., 2011). The human H2 and H4 receptors and the rat H3 receptor isoforms, as well as surprising selectivity for H3 receptors over other GPCRs.

Cellular Proliferation Assays. R-SAT assays were performed as described (Burstein et al., 2011). The data for human and rat H3 receptors and human H2 and H4 receptors were generated as follows. Cells were plated 1 day before transfection using 7 · 10⁶ cells in 0.1 ml of media per well of a 96-well plate. Cells were transiently transfected with 1–10 ng/well receptor DNA, and 30 ng/well pSIP-β-galactosidase (Promega Corporation) per well of a 96-well plate using Polyfect (Qiagen, Valencia, CA) or Sigma/RBI (St. Louis, MO).

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Membrane Preparations. Membranes were made as previously reported (Burstein et al., 2011) with the following modifications. HEK-293T cells were seeded at 13.5 · 10⁶ cells per 15-cm dish and were transfected 24 hours later by mixing 11 µg of DNA in 900 µl of

Fig. 1. Structures of amiodarone, lorcaidine, and other H3 antagonists. The other H3 antagonists shown have been discussed (see Berlin et al., 2011; Lebois et al., 2011; and Singh and Jadhav, 2013).
DMEM, adding 33 μl of FuGENE 6 (Roche Applied Science, Indianapolis, IN) dropwise, incubating the mixture for 15 minutes at room temperature, and adding it to the plate. Cells were not centrifuged after cell scraping, but were collected directly into the ice-cold nitrogen cavitation chamber.

**Radioligand Binding Assays.** Membranes were prepared as described above. For binding assays, membranes expressing H3 (5 μg/well) or H4 (10 μg/well) were incubated with 0.5 nM [3H]-α-methylhistamine for H3 or 10 nM [3H]histamine for H4 for 1 hour at room temperature in binding buffer 1 (50 mM Tris, 10 mM MgCl2, 1 mM EDTA, 0.1% bovine serum albumin, pH 7.4). Membranes expressing M1 (5 μg/well), M2 (5 μg/well), M3 (8 μg/well), M4 (8 μg/well), and M5 (4 μg/well) were incubated with 0.4 nM [3H]N-methylscopolamine (0.5 nM for M5) for 2 hours at room temperature in binding buffer 2 (25 mM sodium phosphate, 5 mM MgCl2, 0.1% bovine serum albumin, pH 7.4). Binding reactions were terminated by filtration through PerkinElmer UniFilter-96 GF/B filters (presoaked with 0.1% polyethylenimine for 1 hour) with a Brandel 96-well harvester (Brandel, Inc., Gaithersburg, MD). Filters were washed with ice-cold wash buffer (25 mM HEPES, 250 mM NaCl, 1 mM CaCl2, 5 mM MgCl2, pH 7.4, 500 ml/plate) and then allowed to air-dry for overnight. We added 50 μl of MacrosScint-20 scintillation cocktail to each well, and the plates were sealed and then counted 3 hours later on a TopCount NXT (PerkinElmer Life and Analytical Sciences).

**Data Analysis.** Agonist curves from R-SAT, cAMP, and radioligand binding experiments were fitted to a sigmoidal dose-response function: \( Y = B + (T - B)/(1 + 10^{(\text{LogEC}_{50} - \text{Log}X)})^H \), where \( Y \) is the response, \( B \) is the baseline, \( T \) is the top or maximum response, \( H \) is the slope of the curve (Hill slope), and \( X \) is the concentration of ligand. For R-SAT and cAMP assays, \( H \) was set to 1. Calculation of \( K_i \) values in the functional antagonist assays was performed using the Cheng–Prusoff method (Cheng and Prusoff, 1973). All data analysis was performed using GraphPad Prism software (version 4.0; GraphPad Software Inc., San Diego, CA).

**Results**

We developed a high-throughput cellular proliferation assay that is compatible with most GPCRs and that detects constitutive receptor activity with high sensitivity (R-SAT) (Burstein et al., 2006). To identify novel small molecule ligands, R-SAT was used to screen the human H3 histamine receptor against a collection of more than 200,000 compounds. The compound library included products of combinatorial chemical synthesis, medicinal chemistry synthesis, and more than 2000 clinically used drugs. A large number of novel antagonists and inverse agonists at the H3 receptor were identified that will not be described here.

Among the “hits” identified were two antiarrhythmic drugs, the class IC sodium channel blocker lorcainide, and the class III potassium ion channel blocker amiodarone (Fig. 1). Full concentration-response experiments run with H3 receptors demonstrated that both compounds were potent inverse agonists at human histamine receptors (Fig. 2; Table 1). The inverse
agonist activities of both compounds were most apparent at isoform 2, and less so at the other isoforms tested. The high constitutive activity of isoform 2 of the human H3 receptor is consistent with a previous report (Bongers et al., 2007). Interestingly, the H3 receptor antagonist clobenpropit displayed partial agonist activity, especially at isoforms 2 and 4.

Full concentration-response experiments run with H3 receptors in the presence of histamine demonstrated that amiodarone and lorcainide were potent functional antagonists at H3 histamine receptors (Fig. 3). Compared with H3 receptor reference compounds, both lorcainide and amiodarone were more potent than thioperamide and less potent than clobenpropit or iodophenpropit (Table 1). Clobenpropit only partially suppressed histamine-induced activation of isoform 2, consistent with its partial agonist activity at that isoform. The potencies of amiodarone and lorcainide were similar at isoform 1 of the human H3 receptor and its rat ortholog. In contrast, thioperamide and clobenpropit were approximately 10-fold more potent at rat H3 receptors, consistent with previous reports (Ligneau et al., 2000; Stark et al., 2001; Yao et al., 2003).

H3 receptors couple to Gαi, G-proteins and inhibit production of cAMP (Lovenberg et al., 1999). cAMP assays were run to confirm results obtained using R-SAT. Both amiodarone and lorcainide were each able to block histamine-induced cAMP inhibition more potently than thioperamide (Fig. 4A; Table 2). Similar to the R-SAT results, both lorcainide and amiodarone displayed inverse agonist activity, apparent as increases in cAMP levels above baseline (Fig. 4B; Table 2). Clobenpropit also had inverse agonist activity in cAMP assays, despite showing partial agonist activity in the cellular proliferation assays.

Recently amiodarone was reported to bind allosterically to muscarinic acetylcholine receptors (Stahl and Ellis, 2010; Stahl et al., 2011). Therefore, we tested lorcainide and amiodarone in radioligand binding assays to H3 and H4 histamine receptors and all five subtypes of muscarinic acetylcholine receptors. Both lorcainide and amiodarone were able to fully displace 3H]N-acetyl histamine from both human and rat H3 receptors (Fig. 5). In contrast, at H4 receptors, only amiodarone could completely displace the radioligand, and only at much higher concentrations. Similarly, neither drug was able to fully displace 3H]N-methyl scopolamine from any of the muscarinic receptor subtypes. Amiodarone and lorcainide were slightly more potent at human H3 receptors in the binding assays, whereas the reference antagonists, especially thioperamide, were more potent at rat H3 receptors (Table 3).

We profiled amiodarone and lorcainide in R-SAT at a collection of 23 other monoaminergic GPCRs, including the other histamine receptor subtypes, the five muscarinic receptor subtypes, the D2 dopamine receptor, five α adrenergic receptor subtypes, and nine serotonergic receptor subtypes (Table 4). The profiles were conducted at each receptor alone (agonist/inverse agonist mode) and in the presence of the relevant reference agonist for each receptor (agonist mode). Weak agonist activity of amiodarone at H2 histamine receptors and weak antagonist activity of amiodarone at M2 muscarinic and 5-HT2B serotonin receptors and lorcainide at M2 muscarinic receptors was observed. None of these interactions at other GPCRs for either amiodarone or lorcainide were comparable in terms of potency to their interactions with H3 histamine receptors.

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**Table 1**

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<tr>
<th>Ligand</th>
<th>Isoform 1</th>
<th>Isoform 2</th>
<th>Isoform 4</th>
<th>Rat H3</th>
<th>Antiagonist/Inverse Agonist</th>
<th>Agonist</th>
<th>K_i (nM)</th>
<th>EC50 (nM)</th>
<th>Inhibition</th>
<th>%S.E.M.</th>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>7.6</td>
<td>64.9</td>
<td>78.6</td>
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<tr>
<td>Clobenpropit</td>
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<td>-</td>
<td>3</td>
<td>-</td>
<td>8.1</td>
<td>48.5</td>
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<tr>
<td>Thioperamide</td>
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<td>4</td>
<td>-</td>
<td>8.1</td>
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<td>97.2</td>
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<td>8.1</td>
<td>37</td>
<td>97.2</td>
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**Table 2**

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The potent interactions with H3 receptors were unique to amiodarone and lorcainide among the antiarrhythmic drugs we tested. There were no significant interactions with H3 receptors seen for 18 other compounds spanning the six major pharmacological classes of antiarrhythmic drugs (Table 5). Dronedarone was recently developed to be a safer alternative to amiodarone (Adlan and Lip, 2013); however, despite its structural similarity to amiodarone, dronedarone had little to no affinity for H3 receptors (Table 5).

Discussion

We used a high-throughput functional screen to identify the antiarrhythmic drugs lorcainide and amiodarone as potent H3 histamine receptor inverse agonists. Using two different functional assays and radioligand binding assays, we showed that lorcainide and amiodarone could block histamine and R-α-methyl histamine–induced functional responses, reduce basal or constitutive activity of H3 receptors, and displace [3H]N-α-methyl histamine binding to H3 histamine receptors. In contrast, none of the 17 other antiarrhythmic drugs tested showed any appreciable activity at H3 receptors. The antagonist/inverse agonist actions of lorcainide and amiodarone were more potent than the reference ligands thioperamide, iodophenpropit, and clobenpropit, which were each approximately 3- to 10-fold more potent at rat H3 receptors. The inverse agonist activities of amiodarone and lorcainide were least apparent at isoform 4, which in addition to the 80-amino acid intracellular loop 3 deletion, also has a much shorter, alternatively spliced C terminus compared with isoforms 1 and 2 [see Wellendorph et al. (2002)].

Compared with their potencies at their “targets,” the potencies of amiodarone and lorcainide at H3 histamine receptors are remarkably high. For example, amiodarone binds to human ether-a-go-go–related protein channels with an affinity of 220 nM (Waldhauser et al., 2008), displaces [3H]nitrendipine binding to guinea pig cerebral cortex membranes with an IC50...
of 97 nM (Nokin et al., 1986), inhibits fast inward Na\(^+\) currents in human atrial cells at 3 to 30 μM concentrations (Lalevée et al., 2003), and inhibits L-type Ca\(^{2+}\) channels expressed in \textit{Xenopus laevis} oocytes at 10 μM concentrations (Ding et al., 2001). Similarly, 200 μM lorcainide displaces approximately 50% of \(^3\)Houabain binding to the Na\(^+\)-K\(^+\) ATPase in guinea

![Diagram](image-url)

**Fig. 5.** Binding of amiodarone and lorcainide to H3, H4, and muscarinic receptors. Radioligand binding studies were conducted as described in \textit{Materials and Methods} using human H3 receptors (isoform 1) and \(^3\)HNAMH (A), rat H3 receptors (ortholog to isoform 1) and \(^3\)HNAMH (B), human H4 receptors and \(^3\)Hhistamine (C), human M1 muscarinic receptors and \(^3\)HNMS (D), human M3 muscarinic receptors and \(^3\)HNMS (E), and human M5 muscarinic receptors and \(^3\)HNMS (F) together with the indicated concentrations of amiodarone (triangles) and lorcainide (inverted triangles). Reference ligands (open circles) were histamine in (A) and (B), thioperamide in (C), and atropine in (D) through (F). Responses were normalized to the reference ligands in each case. NAMH, \textit{N}-α-methyl histamine; NMS, \textit{N}-methyl scopolamine.
Amiodarone is a class III antiarrhythmic agent, used to treat cardiac arrhythmias, but their specific therapeutic uses, mechanisms of action, and attendant side effects are quite different. Amiodarone is a class III antiarrhythmic drug with broad spectrum effects, and effects on adrenergic receptors, and blocker effects. It has also been called a “broad spectrum” antiarrhythmic drug with actions that are similar to those of antiarrhythmic classes IA, II, and IV, including β blocker-like effects, and effects on sodium channels (Punnam et al., 2010). The β blocker effects may be due to downregulation of β-adrenergic receptors, and not a consequence of direct receptor-ligand interaction (Drovota et al., 1999), although low affinity (micromolar) interactions with β receptors have been reported (Schnabel et al., 1999). We observed no significant interactions with β-adrenergic receptors (unpublished observations). Amiodarone causes a number of side effects, particularly on thyroid function due to its chemical similarity to thyroxine, possibly by antagonizing T3 receptor.

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Cellular proliferation assays were run using amiodarone, lorcainide, and the indicated reference agonists and antagonists to obtain pEC₅₀ and efficacy percent values, and pKᵢ and inhibition percent values, respectively. The antagonist assays evaluated the abilities of amiodarone, lorcainide or the relevant reference antagonist to block responses to fixed concentrations (approximately the EC₅₀ concentration) of the relevant reference agonist. Agonist efficacy is normalized to the efficacy of the indicated reference agonists. Antagonist inhibition is normalized to the inhibition of the indicated reference antagonists. The dash indicates that the inhibition constant could not be estimated. ND, not done.

<table>
<thead>
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<tr>
<td>Radioligand binding activity of amiodarone and lorcainide at histamine and muscarinic receptors</td>
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<tr>
<td>Data are presented as the average values for two or more independent experiments. Antagonist potency is reported as the negative logarithm of the IC₅₀ (pIC₅₀).</td>
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<td>Data were fitted using Prism software as described in Materials and Methods. The pIC₅₀ values for the reference ligands were 6.8 for thioperamide at H₄ receptors, and 8.0, 8.2, 7.9, 8.0, and 8.6 at M₁-M₅ receptors, respectively. The human H₃ receptor was isofrom 1 and the rat H₃ receptor was the ortholog to isofrom 1 of the human H₃ receptor.</td>
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<th>Table 4</th>
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<tr>
<td>Profile of amiodarone and lorcainide at 23 other GPCRs in cellular proliferation assays</td>
</tr>
<tr>
<td>Agonist potency is reported as the negative logarithm of the EC₅₀ (pEC₅₀), whereas antagonist potency is reported as the negative logarithm of the Kᵢ (pKᵢ).</td>
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binding to thyroid hormone receptor (Drvota et al., 1995). In addition, amiodarone causes significant peripheral organ toxicity, including liver toxicity, corneal deposits, pulmonary fibrosis, and peripheral neuropathies, but no significant central nervous system side effects (Latini et al., 1984; Santangeli et al., 2012). The peripheral side effects and lack of central nervous system side effects are consistent with the known tissue distribution of amiodarone to liver, lung, heart, and adipose tissue but limited uptake into brain (Riva et al., 1982).

Lorcainide (lorcaainide hydrochloride) is a class IC antiarhythmic agent that works by blocking open fast acting voltage-gated sodium channels (subtype Na1,1.5) (Amery et al., 1981; Eiriksson and Brogden, 1984). Lorcainide is used to help restore normal heart rhythm and conduction in patients with premature ventricular contractions, ventricular tachycardia, and Wolff-Parkinson-White syndrome (Amery et al., 1981; Eiriksson and Brogden, 1984; Winkle et al., 1984). Compared with amiodarone, lorcainide distributes better to the brain (Klotz and Golbs, 1980) and lorcainide usage is less associated with an increased prevalence of central nervous system effects, especially disturbed sleep (see Eiriksson and Brogden, 1984). Interestingly, insomnia is a reported side effect of H3 antagonists in humans (Herring et al., 2012; Othman et al., 2013), and H3 antagonists are in development as treatments for narcolepsy (Inocente et al., 2012). The H3 antagonist activity of lorcainide possibly contributes to its effects on sleep in humans.

Pharmacophores for H3 antagonists have been studied extensively (Berlin et al., 2011; Lebois et al., 2011; Singh and Jadhav, 2013). Structurally, it is possible to rationalize the H3 antagonist/inverse agonist activities of lorcainide and amiodarone. The classic H3 ligands, both agonists (e.g., N\textsubscript{α}-methylhistamine and R\textsubscript{α}-methylhistamine) and antagonists (e.g., thioperamide and clobenpropit), contain an imidazole functionality (see Fig. 1). However, new pharmacophores for H3 antagonists in which the imidazole is replaced by other basic groups are now common. Typically, the basic groups are cyclic amines (e.g., piperidines or pyrrolidines). Certain structural fragments such as alkylamino-alkoxyphenyl motifs (most frequently dialkylamino-propanoxyphenyl) are found in numerous series (see Fig. 1 for examples). The diethylamino-ethoxy-diodophenyl fragment of amiodarone is consistent with this theme, with the ethoxyphenyl spacing a novel variation compared with many of the recently described H3 antagonists. The more linear nature of amiodarone is also reminiscent of both classic and recent examples of H3 antagonists. The diido substitutions in amiodarone are unique and are not seen in any other H3 antagonists; however, interestingly, the marine natural product and H3 antagonist aplysamine contains dibromo substitutions in analogous positions (see Fig. 1). Similarly, the isopropyl-aminopiperidine moiety in lorcainide fits the pattern found in many recent examples of H3 antagonists.

It is interesting to speculate whether the H3 receptor antagonist activities of lorcainide or amiodarone contribute to their antiarrhythmic properties. Functional H3 histamine receptors are expressed in sympathetic nerve endings in human atria, where they have been shown to reduce norepinephrine release such as that which occurs during ischemia/reperfusion injury (Imamura et al., 1996). H3 knockout mice show enhanced norepinephrine release in an ischemic heart model and consequently, increased severity of cardiac arrhythmia (Koyama et al., 2003). These data suggest a cardioprotective role for H3 receptor agonists rather than antagonists; thus, the H3 antagonist activities of lorcainide and amiodarone probably do not contribute to their therapeutic effects. This is consistent with the observation that no other antiarrhythmic drug tested had H3 receptor antagonist activity.

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Authorship Contributions

Participated in research design: Del Tredici, Piu, Burstein. Conducted experiments: Del Tredici, Ma, Burstein. Performed data analysis: Del Tredici, Ma, Burstein. Wrote or contributed to the writing of the manuscript: Burstein.

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